

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
6 October 2005 (06.10.2005)

PCT

(10) International Publication Number
WO 2005/092015 A3

(51) International Patent Classification:

C12P 7/06 (2006.01) C12N 9/28 (2006.01)
C12P 1/04 (2006.01) C12N 9/00 (2006.01)
C12P 1/06 (2006.01)

(21) International Application Number:

PCT/US2005/009218

(22) International Filing Date: 18 March 2005 (18.03.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/554,615 19 March 2004 (19.03.2004) US
60/575,133 28 May 2004 (28.05.2004) US

(71) Applicants (for all designated States except US):
NOVOZYMES NORTH AMERICA, INC [US/US]; 77
Perry Chapel Church Road, Franklinton, North Carolina
27525 (US). NOVOZYMES A/S [DK/DK]; Krogshoejvej
36, DK-2880 Bagsvaerd (DK).

(72) Inventors; and

(75) Inventors/Applicants (for US only): BHARGAVA,
Swapnil [IN/US]; 13049 Townfield Drive, Raleigh,
North Carolina 27614 (US). BISGARD-FRANTZEN,
Henrik [DK/DK]; Elmevaenget 8B, DK-2880 Bagsvaerd
(DK). FRISNER, Henrik [DK/DK]; Ronnebaerhaven 20,
DK-3500 Vaerlose (DK). VIKSO-NIELSEN, Anders
[DK/DK]; Lindevej 12, Jorlunde, DK-3550 Slangerup
(DK). JOHAL, Malcolm [US/US]; 3308 Eagle Trail,
Raleigh, North Carolina 27615 (US).

(74) Agent: NOVOZYMES NORTH AMERICA, INC; 500
Fifth Avenue, Suite 1600, New York, New York 10110
(US).

(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,
GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,
KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,
MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG,
PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY,
TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU,
ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,
FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO,
SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN,
GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments

(88) Date of publication of the international search report:
27 July 2006

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: LIQUEFACTION PROCESS

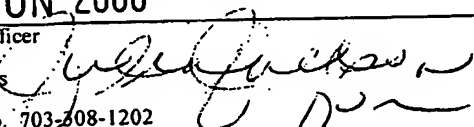
(57) Abstract: The present invention relates to method of liquefying starch-containing material, wherein the method comprises the steps of (a) treating the starch-containing material with a bacterial alpha-amylase at a temperature around 70-90° C for 15-90 minutes, and (b) treating the material obtained in step (a) with an alpha-amylase at a temperature between 60-80° C for 30-90 minutes. The invention also relates to a process of producing a fermentation product, preferably ethanol, comprising a liquefaction step carried out according to the liquefaction method of the invention.

WO 2005/092015 A3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US05/09218

A. CLASSIFICATION OF SUBJECT MATTER IPC(8): C12P 7/06(2006.01);1/04, 06;C12N 9/28(2006.01) C12N 9/00(2006.01) USPC: 435/161,169,170,183,201 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 435/161,169,170,183,201 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Please See Continuation Sheet		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 6,184,002 B1 (MITCHINSON et al) 06 February 2001 (06.02.2001), abstract, columns 1-5	1-12,17 ----- 1-32
Y	US 4,316,956 (LUTZEN) 23 February 1982 (23.02.1982), abstract, columns 1-5, examples	1-32
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents:		
"A"	document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier application or patent published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed	
Date of the actual completion of the international search 11 May 2006 (11.05.2006)		Date of mailing of the international search report 06 JUN 2006
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201		Authorized officer Ruth A. Davis  Telephone No. 703-208-1202

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US05/09218

Continuation of B. FIELDS SEARCHED Item 3:

WEST, PubMed, STN-CAS

search terms: liquefying starch, amylase, bacterial, fermentation, ethanol, wet mill, dry mill, cereal

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44bis)

Applicant's or agent's file reference 10620.204-WO	FOR FURTHER ACTION	See item 4 below
International application No. PCT/US2005/009218	International filing date (<i>day/month/year</i>) 18 March 2005 (18.03.2005)	Priority date (<i>day/month/year</i>) 19 March 2004 (19.03.2004)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant NOVOZYMES NORTH AMERICA, INC		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 bis.1(a).
2. This REPORT consists of a total of 5 sheets, including this cover sheet.

In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

- | | |
|---|---|
| <input checked="" type="checkbox"/> Box No. I | Basis of the report |
| <input type="checkbox"/> Box No. II | Priority |
| <input type="checkbox"/> Box No. III | Non-establishment of opinion with regard to novelty, inventive step and industrial applicability |
| <input type="checkbox"/> Box No. IV | Lack of unity of invention |
| <input checked="" type="checkbox"/> Box No. V | Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement |
| <input type="checkbox"/> Box No. VI | Certain documents cited |
| <input type="checkbox"/> Box No. VII | Certain defects in the international application |
| <input type="checkbox"/> Box No. VIII | Certain observations on the international application |

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44bis.3(c) and 93bis.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44bis .2).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Date of issuance of this report 19 September 2006 (19.09.2006)
	Authorized officer Simin Baharlou e-mail: pt09@wipo.int

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

REC'D 08 JUN 2006

PCT

PCT

To:
JASON I. GARBELL
NOVOZYMES NORTH AMERICA, INC.
500 FIFTH AVENUE, SUITE 1600
NEW YORK, NY 10110

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

Date of mailing (day/month/year) 06 JUN 2006	
FOR FURTHER ACTION See paragraph 2 below	
Applicant's or agent's file reference 10620.204-WO	
International application No. PCT/US05/09218	International filing date (day/month/year) 18 March 2005 (18.03.2005)
Priority date (day/month/year) 19 March 2004 (19.03.2004)	
International Patent Classification (IPC) or both national classification and IPC IPC(8): Please See Continuation Sheet USPC: 435/161,169,170,183,201	
Applicant NOVOZYMES NORTH AMERICA, INC.	

1. This opinion contains indications relating to the following items:

- | | | |
|-------------------------------------|--------------|--|
| <input checked="" type="checkbox"/> | Box No. I | Basis of the opinion |
| <input type="checkbox"/> | Box No. II | Priority |
| <input type="checkbox"/> | Box No. III | Non-establishment of opinion with regard to novelty, inventive step and industrial applicability |
| <input type="checkbox"/> | Box No. IV | Lack of unity of invention |
| <input checked="" type="checkbox"/> | Box No. V | Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement |
| <input type="checkbox"/> | Box No. VI | Certain documents cited |
| <input type="checkbox"/> | Box No. VII | Certain defects in the international application |
| <input type="checkbox"/> | Box No. VIII | Certain observations on the international application |

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/ US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201	Date of completion of this opinion 11 May 2006 (11.05.2006)	Authorized officer Ruth A. Davis Telephone No. 703-308-1202
--	---	---

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US05/09218

Box No. I Basis of this opinion

1. With regard to the language, this opinion has been established on the basis of:

- ☒ the international application in the language in which it was filed
- ☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

a. type of material

- ☒ a sequence listing
- ☐ table(s) related to the sequence listing

b. format of material

- ☒ on paper
- ☒ in electronic form

c. time of filing/furnishing

- ☒ contained in the international application as filed.
- ☒ filed together with the international application in electronic form.
- ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US05/09218

Box No. V Reasoned statement under Rule 43 bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims <u>13-32</u>	YES
	Claims <u>1-12</u>	NO
Inventive step (IS)	Claims <u>NONE</u>	YES
	Claims <u>1-32</u>	NO
Industrial applicability (IA)	Claims <u>1-32</u>	YES
	Claims <u>NONE</u>	NO

2. Citations and explanations:

Claims 1 - 12 and 17 lack novelty under PCT Article 33(2) as being anticipated by Mitchinson et al. (US 6184002).

The claims are to a method for liquefying starch wherein the starch is treated at specific temperatures with alpha-amylase. Dependent claims specifically limit the method to specific temperatures, amounts of amylase, time periods and jet cooking. Additional limitations are to the type of starch, i.e. cereals, milling, source of amylase, fermentation steps, and the recovered products.

Mitchinson teaches the claimed steps of liquefying starch by combining cereals with alpha amylase at the claimed temperatures, wherein the cereals are milled in the claimed manner (columns 1 - 5). The reference teaches the claimed source of amylase as well as stating that the sources can be varied according to one in the art.

Claims 1 - 37 lack an inventive step under PCT Article 33(3) as being obvious over Mitchinson et al (US 6184002) in view of Lutzen (US 4316956).

The claims are to a method for liquefying starch wherein the starch is treated at specific temperatures with alpha-amylase. Dependent claims specifically limit the method to specific temperatures, amounts of amylase, time periods and jet cooking. Additional limitations are to the type of starch, i.e. cereals, milling, source of amylase, fermentation steps, and the recovered products.

Mitchinson teaches the claimed steps of liquefying starch by combining cereals with alpha amylase at the claimed temperatures, wherein the cereals are milled in the claimed manner (columns 1 - 5). The reference teaches the claimed source of amylase as well as stating that the sources can be varied according to one in the art.

Mitchinson does not specifically teach recovering ethanol, or steps for practicing such steps. However, Mitchinson does teach that ethanol can be recovered by steps known in the art (col.1-5). Lutzen teaches the claimed steps of obtaining ethanol, wherein the claimed yeast is used to ferment the sugars obtained from the process of starch liquefaction (col. 1-5). Thus, at the time of the claimed invention, one of ordinary skill in the art would have been motivated by the cited reference to practice the claimed invention with a reasonable expectation for success.

Claims 1 - 32 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US05/09218

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of IPC:
C12P 7/06(2006.01);C12P 1/04(2006.01),1/06(2006.01);C12N 9/28(2006.01)
C12N 9/00(2006.01)